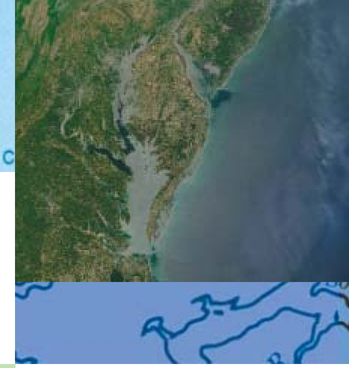

NOAA CHESAPEAKE BAY OFFICE

Non-native Oyster Research

Research Topics:

Understanding Crassostrea ariakensis within its native range

Susceptibility to disease-causing parasites and pathogens

Human consumption risk

Ecology and potential for population growth and sustainability

Interactions with native oyster species

Potential to become a fouling nuisance

Ecosystem services and functions

NOAA CHESAPEAKE BAY

Quarterly Review
Spring 2005

Introduction

Decline in abundance of the native oyster, *Crassostrea virginica*, in the Chesapeake Bay has lead to the collapse of a formerly productive fishery and the loss of significant ecological services. Two oyster diseases, MSX and Dermo, have contributed at least in part to the decline, and continue to challenge oyster restoration efforts. In response to this situation the states of Maryland and Virginia have proposed to intentionally introduce a non-native oyster species, *Crassostrea ariakensis*, which has greater resistant to the pathogens responsible for MSX and Dermo. Considerable controversy exists over the proposed course of action and many questions remain about the implications of such an introduction. In 2003 the U.S. Congress mandated that an Environmental Impact Statement (EIS) be prepared to examine both the risks and benefits of introducing this species to the Chesapeake Bay. The EIS is being conducted by the Army Corps of Engineers as the lead federal agency, with the states of Maryland and Virginia serving as lead state agencies. The U.S. Environmental Protection Agency (EPA), National Oceanic and Atmospheric Administration (NOAA), and Fish & Wildlife Service (FWS) are cooperating agencies on the EIS.

In 2004 the NOAA Chesapeake Bay Office (NCBO) initiated a research program funded at $2M annually to support the scientific information needs of the EIS. The program is aimed at research priorities recently identified by the National Research Council (NRC) and the Scientific and Technical Advisory Committee of the Chesapeake Bay Program (STAC), as well as guidance from the International Code of Practice on the Introductions and Transfers of Marine Organisms (ICES Code of Practice). Quarterly review sessions will facilitate timely discussions of research results among scientists and managers, and speed the transfer of information to the EIS evaluation process.

The first quarterly review session was held on March 10, 2005 as a webconference sponsored by NCBO and hosted by the Chesapeake Research Consortium. Invitees included scientists conducting research relevant to the oyster EIS, and representatives from federal and state agencies and other interested management groups. This report summarizes the preliminary findings presented during the review session. Several projects conducted in the past or currently underway were not presented, so the review session cannot be viewed as fully comprehensive of all the relevant information. Also, it must be emphasized that the findings of ongoing research are <u>preliminary</u>, and additional time will be required for the projects to be completed before they can be expected to yield final results. Similar quarterly review sessions are planned for the future, and will likely focus on specific areas of research with significant new findings or topics not covered in this first review session (e.g., economics, modeling).

I. Understanding *Crassostrea ariakensis* within its native range: Taxonomy, population genetics, ecology, and pathogens

<u>Why is this research important for the EIS?</u>

Recent molecular work has indicated that *C. ariakensis* (recently synonymized with *C. rivularis*) may not be a single species, and there has been general confusion regarding the taxonomic identification of *Crassostrea* species in Asia. Resolving the taxonomy of *C. ariakensis* in its native range is necessary to determine how much of the existing scientific literature on "*C. ariakensis*" or "*C. rivularis*" can be reliably utilized in EIS evaluations. Knowing the true species boundaries is also a necessary foundation for understanding many other aspects of *C. ariakensis* biology.

The genetic composition of the *C. ariakensis* brood stock that would be propagated by hatcheries to implement the proposed introduction may be a significant factor in determining the long-term success of the species in novel environments outside of its native range where *C. ariakensis* evolved. All *C. ariakensis* currently in the U.S. are known to be derived from a dozen or fewer *C. ariakensis* brood stock, such that genetic bottlenecks and reduced genetic diversity are major concerns currently under investigation. Population genetics studies may enable us to ascertain the geographic location of the source population for the brood stock, which would permit the local environmental conditions to be studied and compared with those of Chesapeake Bay and other coastal U.S. waters.

The International Council for the Exploration of the Sea (ICES) *2003 Code of Practice on the Introductions and Transfers of Marine Organisms* includes a recommended procedure for evaluating proposed introductions of non-native species prior to reaching a decision on new introductions. The United States is a signatory to this international Code of Practice. The ICES Code of Practice recommends understanding the ecology, genetics, and diseases within the native range of a species before reaching a decision to introduce that species elsewhere.

Presentations:

Jan Cordes (VIMS) – Assessing levels of genetic variation within and among native populations and hatchery stocks of the Suminoe oyster *C. ariaken*sis using a suite of molecular markers.

Ximing Guo (Rutgers) – Genetic and ecological structures of *C. ariakensis* estuaries in China.

David Bushek (Rutgers) – A histological investigation of oyster parasites and pathology in three Chinese estuaries containing varying mixtures of coexisting oyster species, including *C. ariakensis*.

Some preliminary findings:

- The work to date has resulted in the development of diagnostic molecular tools. These tools are now beginning to be applied to answer questions about *C. ariakensis* in its native range, and about the genetic composition of the *C. ariakensis* brood stock held in the U.S.
- The development of these molecular tools represents a major advance because we are now able to discriminate more confidently among the *Crassostrea* species in Asia. In addition to the traditionally recognized *Crassostrea* species in Asia, new species are now being discriminated and identified.
- There are two species of *Crassostrea* that have been called *C. ariakensis* in the past literature. These two species are now described as *C. ariakensis* and *C. hongkongensis*. *Crassostrea ariakensis* was also misidentified as *C. gigas* in some literature. The true *C. ariakensis* species includes two genetically distinct strains: a southern strain and a northern strain. With this revised description of *C. ariakensis*, we still do not have a good understanding of the distribution and abundance of *C. ariakensis* across its native range. Over the next year, researchers will conduct expeditions to collect oysters from specific locations in China and Japan to fill geographic gaps from previous sampling efforts.
- There is no genetic evidence of hybridization in the wild between *C. ariakensis*, *C. hongkongensis*, *C. gigas*, or other Asian *Crassostrea* species identified so far, but this

preliminary finding is based only upon the limited number of individuals that have been genetically analyzed to date. Laboratory experiments are underway to determine whether *C. ariakensis* can hybridize with *C. virginica* under a range of environmental conditions typical of Chesapeake Bay and other coastal estuaries along the eastern seaboard.

- Most *C. ariakensis* individuals held at various laboratories in the mid-Atlantic region (VIMS, HSRL, and UMCES AREL) derived from *C. ariakensis* oysters that were accidentally brought to Oregon with a shipment of Kumamoto oysters (*C. sikamea*) in the early 1990s. The progeny from these oysters are referred to as the "Oregon strain" *C. ariakensis* (also known as WCA, West Coast *ariakensis*). All laboratory and field experiments currently underway in the mid-Atlantic region are using Oregon strain *C. ariakensis*. The Oregon strain has been identified as the northern strain of the true *C. ariakensis* species.
- The "Oregon strain" has reduced genetic variability relative to wild populations of *C. ariakensis*. A quantitative estimate of this reduction in genetic diversity will likely be available by early 2006. Current studies underway will also reveal whether there is variation among the Oregon strain brood stocks held at different hatcheries within the U.S.

II. Susceptibility of *C. ariakensis* to disease-causing parasites and pathogens/Human consumption risk

Why is this research important for the EIS?

C. ariakensis is being investigated for its susceptibility to, and potential to serve as a vector for, a range of waterborne pathogens. A few examples are given in the next paragraphs. This research is important because it will allow us to determine whether the oyster and human disease risks presented by *C. ariakensis* are greater or lesser than those of native bivalve species.

Perkinsus marinus causes the disease Dermo in oysters, but does not affect humans. Dermo is one of the two oyster diseases having major impacts on native oyster populations in Chesapeake Bay and elsewhere along the east coast. Questions relevant to the EIS include the degree to which *C. ariakensis* is susceptible to *Perkinsus marinus* and whether *C. ariakensis* could serve as a disease vector to further exacerbate the impact of this pathogen on native *C. virginica* populations.

Bonamia, an oyster pathogen in other parts of the world, was unknown from the mid-Atlantic region until 2003 when mass mortalities of triploid *C. ariakensis* were observed in experimental deployments in North Carolina. Although *Bonamia* infections have not been found in triploid *C. ariakensis* deployed in Chesapeake Bay to date, it is possible that the pathogen would eventually find its way to the Bay and become established if a suitable host (*C. ariakensis*) were present. We need to determine the geographic distribution and environmental tolerances of the *Bonamia* species discovered in North Carolina, in order to understand how *C. ariakensis* may be limited by *Bonamia* at salinities and temperatures characteristic of Chesapeake Bay and other mid-Atlantic coastal waters.

Viruses are also of concern, and are generally more difficult to study than other pathogens. Viruses can have long latency periods, and identification of viruses requires molecular assays. Some viruses may affect humans, while others are only harmful to oysters. For example, the oyster herpes virus does not infect humans, but can be a major problem in oyster hatcheries. Key issues for investigation include the detection of viruses, the susceptibility of *C. ariakensis* to various known viruses, and the potential transmission of viruses from oyster parents to offspring despite standard quarantine procedures.

Human illness from the consumption of raw oysters is a risk which is proactively mitigated in the U.S. by food safety practices including water monitoring, shellfish harvest closure areas, and post-harvest handling standards. These regulations and standards have been developed from research and experience with shellfish species already present in the U.S. If an introduced shellfish species concentrates toxins or human-illness causing pathogens at a greater rate, or purges these materials from their tissues at a slower rate than native species, the existing regulations and standards may not be adequate to protect human health.

Presentations

Yonathan Zohar (UMBI/COMB) – Overview of research and development studies on *C. ariakensis* at COMB.

Eric Schott, Jose-Antonio Robledo (for Gerardo Vasta, UMBI/COMB) – Assessment of resistance of *C. ariakensis* to Dermo.

Feng Chen (UMBI/COMB) – Risk assessment of viral pathogens.

Kimberly Reece (VIMS) – Potential for *C. ariakensis* to serve as a vector for exotic pathogens in Chesapeake Bay: Assessing the risk of Asian *Perkinsus* spp. and oyster herpes virus to *C. ariakensis* and native bivalves.

Eugene Burreson (VIMS) – Bonamia in *C. ariakensis* in North Carolina

Thaddeus Graczyk (JHU) – Environmental tolerance-dependent competition between adult *C. ariakensis* and *C. virginica* in recovering and retaining waterborne disease agents in relation to water salinity.

Some preliminary findings:

Perkinsus

- *C. ariakensis* in Asia has been found to harbor two *Perkinsus* species.
- *C. ariakensis* does acquire infections of *Perkinsus marinus*, the *Perkinsus* species that causes Dermo disease in oysters of Chesapeake Bay and other coastal U.S. waters. The prevalence of *P. marinus* in *C. ariakensis* host cells does not drop during maintenance in aquaculture, which means that *C. ariakensis* is not clearing the parasite. In vitro, *C. ariakensis* hemocytes do not efficiently kill *P. marinus*. We do not know the potential for *C. ariakensis* to transmit *P. marinus* to the native *C. virginica*.
- Based upon all the field studies to date (5-6 years), there is no evidence that *C. ariakensis* is killed by MSX or Dermo. This finding is limited to field tests of triploids in aquaculture growing conditions (typically not on-bottom).
- We should test *C. ariakensis* infected with *Perkinsus marinus* under stressful conditions that are typical in Chesapeake Bay (low dissolved oxygen, freshets, high sedimentation, etc.). In general, we need to carry out disease studies in combination with ecological studies conducted under stressful conditions representative of the Chesapeake Bay environment.

Bonamia

- Two *Bonamia* species have now been discovered in North Carolina. Only one of the two *Bonamia* species infects *C. ariakensis*, and was responsible for the mass mortalities observed in 2003. To date, this *Bonamia* species has only been found in the higher salinity waters around Bogue Sound, North Carolina. This species is most closely related to *Bonamia* species endemic to the southern hemisphere. This preliminary finding raises new questions: How did a southern hemisphere pathogen get to North Carolina, how long has it been here, will the pathogen persist in this new environment, and could it spread to other areas on the East Coast?
- *C. ariakensis* spat (<40mm) are highly susceptible to the southern hemisphere *Bonamia sp*. Larger individuals are less susceptible.
- The southern hemisphere *Bonamia sp.* does not seem to tolerate lower salinity. Field and laboratory studies currently underway will provide more information about the environmental conditions suitable for this pathogen.

Viruses

- *C. ariakensis* in Asia has been found to harbor two strains of the oyster herpes virus. The potential for vertical transmission from parents to progeny under hatchery conditions is currently being investigated.
- No oyster herpes virus has been found in the Oregon strain *C. ariakensis* individuals tested to date.
- *C. ariakensis* must be tested for susceptibility to the viral communities occurring in Chesapeake Bay and other coastal waters.

Human consumption risk

- We do not know if the risk of human illness from consumption of *C. ariakensis* is greater or lesser than that of native bivalve species. Data on the recovery efficiency and pathogen retention rates of *C. ariakensis* for the following human disease-causing waterborne pathogens will be available at the end of 2005 with work continuing into 2006: Noroviruses (Norwalk-like viruses), Hepatitis A virus, and Adenovirus; bacteria such as *Vibrio vulnificus*, *V. parahaemolyticus*, *Yersinia enterocolitica*, *Mycobacterium avium/marinum* complex; and protozoa including *Cryptosporidium parvum*, *Toxoplasma gondii*, *Giardia duodenalis*, and human-infective microsporidia such as *Encephalitozoon intestinalis*, *E. cuniculi*, *E. hellem,* and *Enterocytozoon bieneusi*.

III. Ecology/Interactions between *C. ariakensis* and native oyster species/Fouling potential/ Ecosystem services and functions

<u>Why is this research important for the EIS?</u>

Two analytical tools being developed to help evaluate the proposed action and alternatives are an oyster demographic model and a larval transport model. The accuracy and predictive power of these models will depend upon the quality of data available for model input on a variety of physiological, behavioral, and ecological parameters. Research to identify the mean, range, and variability of these parameters for *C. ariakensis* growing in real-world conditions of U.S. coastal estuaries is necessary for model inputs.

Fouling of man-made structures such as docks, boats, buoys, and water intake structures can result in significant economic costs. The potential for *C. ariakensis* to become a fouling nuisance must be examined and possible long-term costs estimated as part of the cost-benefit analyses of the EIS. A related area of inquiry is the degree to which *C. ariakensis* and *C. virginica* will compete for space on the limited hard bottom substrates available, and whether one species might out-compete and eventually eliminate the other over time.

The native oyster is a prolific builder of reefs that provide important habitat for many other estuarine species; yet not all oysters are reef-builders. Several oyster species are referred to as "rock oysters" because their flat growth form hugs the surface of rocks and other bottom substrates. It is unclear whether *C. ariakensis* forms reefs similar to those of *C. virginica* and, if they do, under what environmental conditions.

In addition to reef habitat formation, another ecosystem service desired of *C. ariakensis* is water filtration to remove excess phytoplankton and sediment resulting in improved water quality and clarity. All oysters are filter-feeders, but there is variation among species in filtration rates and the sizes and types of particles removed from the water during filter feeding. Data on *C. ariakensis* filter-feeding dynamics under varying environmental conditions are needed for input into water quality models to estimate water quality improvements that might reasonably be expected from *C. ariakensis* and *C. virginica* at various population sizes.

<u>Presentations</u>

John Stubblefield (UMBI/ COMB) – Environmental tolerance studies on *C. ariakensis*.

Mark Luckenbach (VIMS) – Behavior, substrate selection, and survival of *C. ariakensis* pediveliger larvae and juveniles to variation in environmental conditions.

Mark Luckenbach (VIMS) – Comparative post-settlement growth and survival in the Suminoe oyster, *C. ariakensis,* exposed to simulated intertidal emersion.

Joan Manuel (UMCES) – Behavioral responses of *C. ariakensis* and *C. virginica* larvae to environmental change under spatially realistic conditions.

Roger Newell (for Vic Kennedy, UMCES) – Predation by polyhaline invertebrate predators on young non-native oysters, *C. ariakensis*, in Chesapeake Bay.

Roger Newell (UMCES) – Competitive interactions between diploid Eastern and diploid Suminoe oysters from larval settlement through to development of reefs and the assessment of the habitat value of such reefs.

Kennedy Paynter (UMD) – Characterizing performance of the Suminoe oyster, *C. ariakensis*, in Maryland waters.

David Bushek (Rutgers) – Fertilization interference between *C. ariakensis* and *C. virginica*.

<u>Some preliminary findings:</u>

- Although *C. ariakensis* can tolerate a wide range of salinities from 5 ppt to 40 ppt, the medium salinity range (10-25 ppt) seems to be optimal for survival and growth. Additional replication is needed before this can be considered a robust conclusion. Salinity tolerance must also be examined for life stages other than adults. Gametes and embryos have not yet been tested by exposure to various salinity regimes.

- Both *C. ariakensis* and *C. virginica* prefer to settle on oyster shell, and both species exhibit the highest rates of settlement on biofouled oyster shell. *C. ariakensis* appears to have a greater propensity for settling on some man-made materials tested to date, specifically fiberglass, compared with *C. virginica*. This finding must be confirmed with additional testing and replication.
- *C. ariakensis* and *C. virginica* have been found to differ in larval behavior and in the distribution of larvae in the water column. *C. ariakensis* larvae have a greater tendency to occur at the bottom, whereas *C. virginica* larvae favor the upper water column. Larvae of both species respond to even slight haloclines (0.25 ppt), and larval responses to haloclines change throughout the day. There is, however, considerable variability in these data, so large datasets with longer time series are needed to better understand complex larval behavior patterns.
- Comparative predation studies with *C. ariakensis* and *C. virginica* spat (<15mm size) exposed to the typical suite of mesohaline predators are finding that both species are equally vulnerable to flatworm predation, but *C. ariakensis* is significantly more vulnerable to mud crab predation.
- In mesocosm studies of diploid oysters growing in single-species tanks there was similar growth of *C. ariakensis* and *C. virginica* during the summer months, but *C. ariakensis* grew significantly faster than *C. virginica* from November to January. Additional time is needed to complete this work and confirm the annual growth pattern for *C. ariakensis* in other years. Furthermore, these findings will be compared to those from the mixed-species (both *C. ariakensis* and *C. virginica*) mesocosm tanks when those data become available.
- The same pattern of active growth in *C. ariakensis* during the winter months has been observed in triploids caged in shallow water field experiments.
- There is evidence that *C. ariakensis* may be more susceptible than *C. virginica* to mortality caused by low dissolved oxygen levels. More work is needed to empirically evaluate this vulnerability.
- Studies underway will provide density-dependent growth rates, quantification of growth form under different density regimes, and competition coefficients (i.e., competition for space) between diploid *C. ariakensis* and *C. virginica* in mesocosms. This information is important for the demographic model, which should include density-dependent terms.
- Across several important physiological and ecological parameters examined to date, *C. ariakensis* appears to be different than *C. virginica*. The importance and effects of these differences are not fully known at the present time. There are also physiological similarities between *C. ariakensis* and *C. virginica*. Both species have evolved to tolerate a wide range of conditions encompassing a variety of environmental parameters. For example, like *C. virginica*, *C. ariakensis* exhibits a tolerance for a wide range of temperature and salinity conditions. We do not yet know about similarities or differences in tolerance for other key environmental parameters such as turbidity and dissolved oxygen.

For information contact:

Jamie L. King, Ph.D.

NOAA Chesapeake Bay Office

410 Severn Avenue, Suite 107A

Annapolis, MD 21403

410-267-5655

Jamie.King@noaa.gov

http://noaa.chesapeakebay.net

Science, Service, Stewardship